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Preparation and Characterization of CuO NPs via Laser Ablation Under Electric Field and Study Their Antibacterial Activity

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Abstract. Antibiotic activity of copper oxide (CuO) nanoparticles (NPs) produced via pulse laser ablation in liquid (PLAL) process with the electric field was investigated. The optical and structural properties for CuO NPs were characterized using FTIR, UV-VIS, and AFM. In case of CuO only, it was found to have an effect on all bacterial species with some variation. However, when NPs are mixed with antibiotics, there is an obvious many difference in the antibacterial activity towards bacteria. This is evidence of a synergistic effect between the two NPs and antibiotics. We found that the percentage of increased inhibitory effect has increased against the Gram-negative bacteria, as in the combination of (Ampicillin and Gentamicin) with NPs (47% and 46%, respectively) against *E. coli* (60%, 67%, 60%, and 150%, respectively) against the intestinal bacteria. In addition, there is a significant increase in the ratio of antibacterial activity against pathogens by the combination of antibiotics (Cefotaxime, Gentamicin, and Ampicillin) with NPs on the growth of *E. coli* bacteria.

INTRODUCTION

Pulsed laser ablation in the liquid environment process (PLAL) has been attracting a lot interest, because its special process to produce nanoparticles (NPs) with different type and morphology. It has been established that NPs producing depending on solvents, laser wavelength, pulse period, and auxiliary laser irradiation of colloids [1–5]. As laser ablation products in partially ionized plasma and then nanoscale size liquid droplets, it is viable to employ electric fields to the plume in the reason to limit the realization of moderately NPs [6]. Metal NPs have individual characteristics that are not available in bulk materials. Uniqueness characteristics of NPs ascribed to their size, structures, and high surface-to-volume ratios (increased the located atoms at the grain boundaries) thus they show significantly novel physical, chemical, and biological properties [7-11]. Antimicrobial agents like metal oxide NPs have been used to inhibit a wide range of microbes such as bacteria and fungi [12]. *S. aureus* is a pathogenic microbe causes a wide range of diseases which includes skin infections, surgical site infections, bacteria and pneumonia [13]. *E. coli* is on the preponderant facultative anaerobes in the human gastrointestinal tract. *E. coli* strains provide health benefits, but may be harmful to the host. A pathogenic *E. coli* can cause diarrheal disease, urinary tract infection, meningitis, and septicemia [7, 8]. Copper oxide (CuO) NPs was utilized as antimicrobial agents due to their effectiveness on resistant strains of pathogens and low toxicity [14]. In this work, prepared CuO NPs via Nd: YAG laser ablation in deionized H_2O in thepresence of a field. The antibacterial activities of CuO NPs with or without different antibiotics of some bacteria were also investigated.

EXPERIMENTAL

Materials and Methods

Preparation of CuO NPs

Copper (Cu) target (99.99%) was utilized as the target material, which fixed on the bottom of the vessel and covered with 2.5 mm of deionizer water, which is used as the liquid environment. The electric field about 5 V/cm was applied during the ablation process. The process was achieved via focused of 1064 nm Nd: YAG laser with 10 ns pulse duration and 1 Hz frequency on the Cu surface, the process is carried out at laser energies (500 mJ/pulse) with 250 numbers of laser pulses. The characterization of these NPs was investigated by using FTIR spectroscopy, UV-VIS spectrometer and atomic force microscope (AFM). These are done at room temperature.

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Antibacterial assay

The agar method was performed in vitro to determine the antibacterial activity of the mixing CuO NPs with antibiotics against several pathogenic bacteria, such as *E. coli*, *K. Pneumonia* (G−ve), and *S. aureus* (G+ve). Pure cultures were swapped with medium. Each strain was uniformly swabbed onto the plates. The antibiotic disk that saturated or immerse with NPs were placed on the dishes by sterile forceps with 3-4 dick. For each Petri dish, then incubated at 37 °C for 24 hours. All samples were tested in triplicate. The diameter was then recorded as the mean ± standard deviation of the triplicate.

RESULTS AND DISCUSSIONS

Figure 1 shows the FTIR spectrum of CuO NPs. The bond of Cu (II) - O was shown at 601 cm^{-1}, 508 cm^{-1}, and 487 cm^{-1}. While the broad absorption at 3430 cm^{-1} due to the adsorbed the water molecules [15]. Figure 2 shows the UV-VIS absorption spectra of the CuO NPs, which seen absorption peaks at 253 nm, and then a slightly shifted (blue) to the present of electric field, implying that the product corresponding to apply electric field has a much smaller size. The absorption peaks of CuO at 251 nm can be used to estimate particle size following effective mass approximation [16, 17].

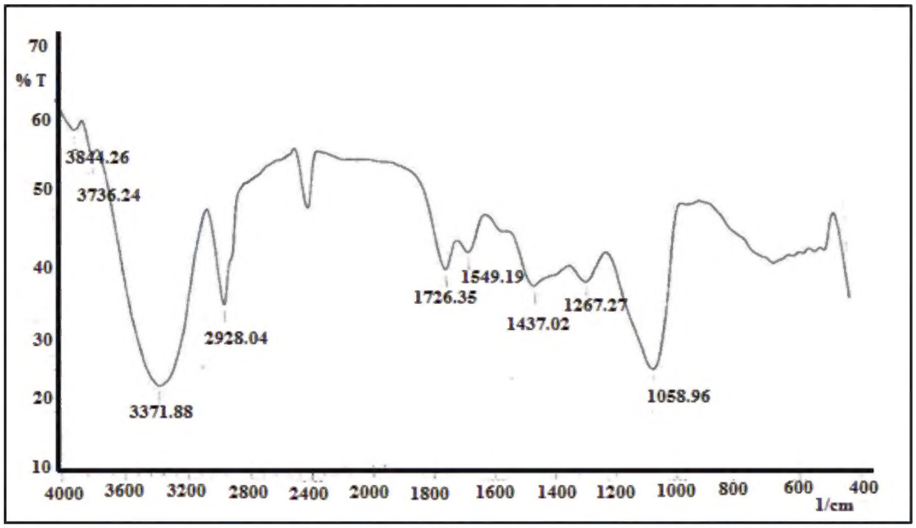

FIGURE 1. The FTIR of the CuO NPs prepared by PLAL method.

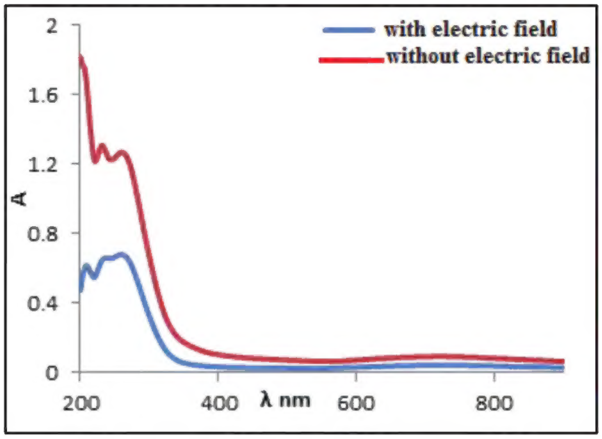

FIGURE 2. The absorption spectra of CuO NPs.

Figures 3 and 4 show the AFM images of CuO NPs prepared via PLAL in the absence and presence of electric field. In general, the prepared particles are small with a spherical shape, and its decrease as an applied external electric field. That causes to decrease the potential energy barrier for Rayleigh disintegration, thus resulting in a decrease in average NP size [6].

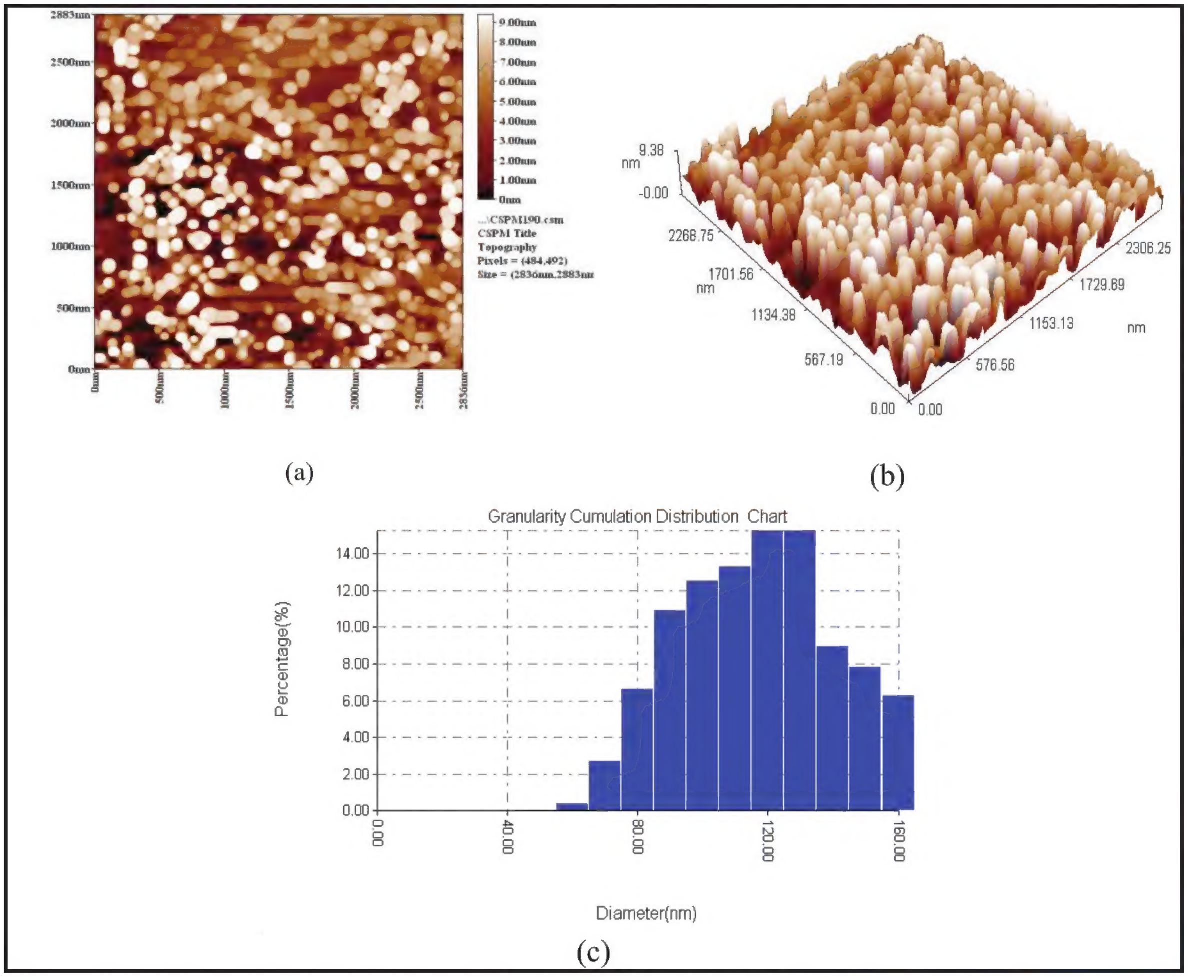

FIGURE 3. (a) 2D and (b) 3D AFM images with (c) particle distribution of CuO NPs prepared via PLAL.

Figures 5 and 6 show the antibacterial activity against some G-*ve* and G+*ve* pathogenic microbe of CuO NPs prepared with and without the presence of electric field, and also shows the effect of combined CuO NPs with different antibiotics. In Fig. 5, notice that the highest effect of CuO NPs on the growth of *S. aureus* has an inhibition zone about 13 mm and 9 mm in *E. coli*, and 7 mm in *K. pneumonia*. The zone of inhibition is determined by measuring the diameter in millimeters. Then, when added the NPs to the antibiotic, notice that there is an increase in the effects on the growth of bacterial strain, these results were shown in Table 1. When the presence of an electric field to prepare CuO NPs the antibacterial activity was shown in Fig. 5, the results exhibit the effect of CuO NPs on the growth of all bacterial strains by inhibition zone 19 mm, 14 mm, and 15 mm, respectively. For the complex of antibiotics with NPs, the highest effect for (AMP+ CuO NPs) in the growth of *K. pneumonia* by inhibition reached until 25 mm and 24 mm in *E. coli*, but there is no effect against *S. aureus*. Also in case of the complex CFX antibiotic with CuO NPs, the highest effect was 25 mm on the growth of *S. aureus*, and 15 mm in *E. coli*, and there is no effect on the growth of *K. pneumonia.* While at Gen. antibiotic we found its effect on the growth of *S. aureus* and *K. pneumonia* by inhibition zone reached to 10 mm, but there is no effect on the growth of *E. coli*, all these results were shown in Table 2.

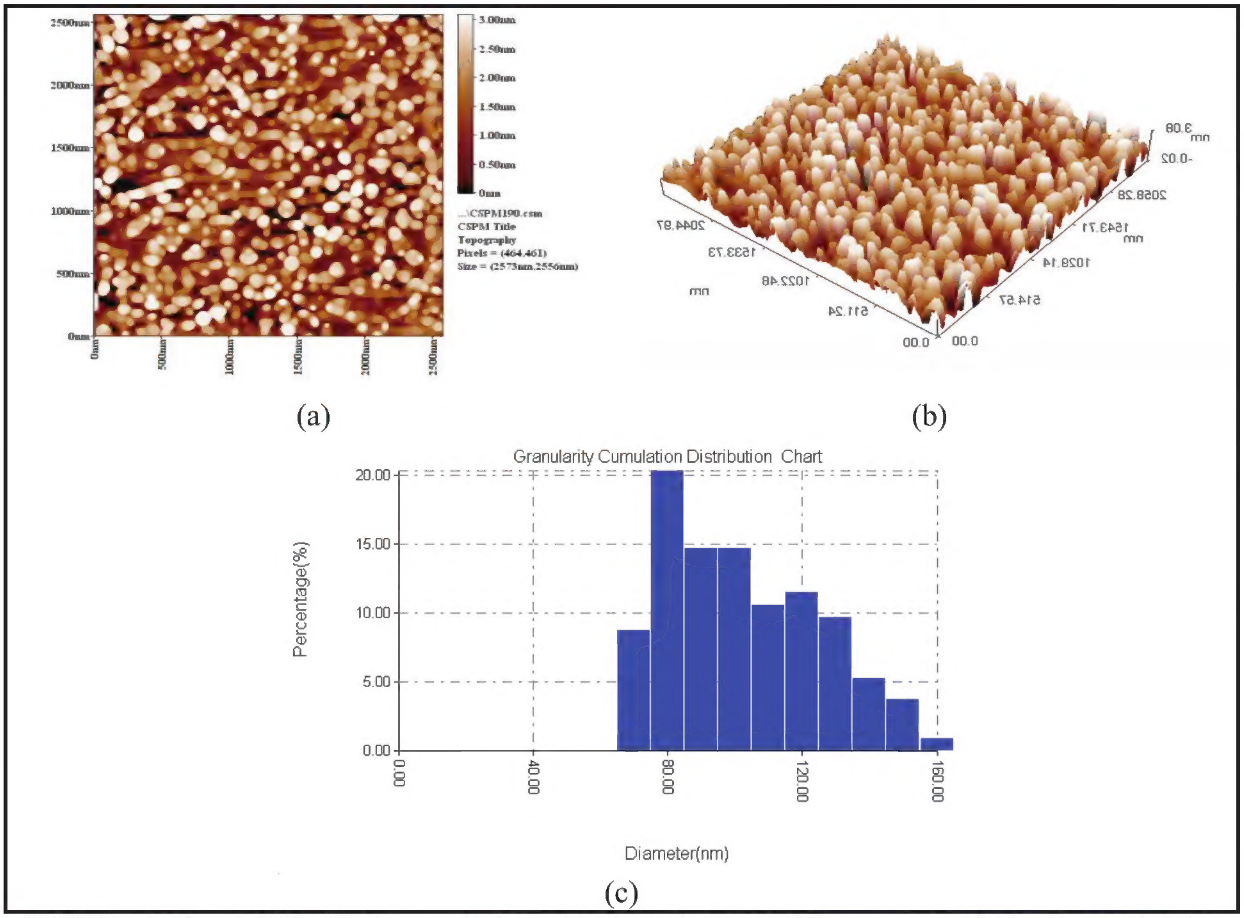

FIGURE 4. (a) 2D and (b) 3D AFM images with (c) particle distribution for CuO NPs prepared via PLAL under electric fields.

TABLE 1. The antibacterial activity of NPs prepared via PLAL with and without different antibiotics against pathogens.

Pathogens	**CuO NPs**	**Antibiotics Zone of inhibition (mm)**								
		Ampicillin (10 µg)		**F.I (%)**	**Cefoxitin (10 µg)**		**F.I (%)**	**Gentamicin (10 µg)**		**F.I (%)**
		Ab	**Ab+NPs**		**Ab**	**Ab+NPs**		**Ab**	**Ab+NPs**	
S. aureus	13	-	-	-	20	23	15	10	13	30
E. coli	7	15	22	47	-	8	33	13	19	46
K. pneumoniae	9	20	24	20	-	-	-	10	12	20

F.I-Fold Increase F= ((b-a)/a) ×100

TABLE 2. The antibacterial activity of NPs prepared via PLAL under electric field with and without different antibiotics against pathogens.

Pathogens	**CuO NPs**	**Antibiotics Zone of inhibition(mm)**								
		Ampicillin (10 µg)		**F.I (%)**	**Cefoxitin (10 µg)**		**F.I (%)**	**Gentamicin (10 µg)**		**F.I (%)**
		Ab	**Ab+NPs**		**Ab**	**Ab+NPs**		**Ab**	**Ab+NPs**	
S. aureus	19	-	-	-	20	25	25	10	15	50
E. coli	14	15	24	60	-	15	150	-	10	67
K. pneumoniae	15	19	25	32	-	-	-	10	14	40

F.I-Fold Increase F= ((b-a)/a)×100

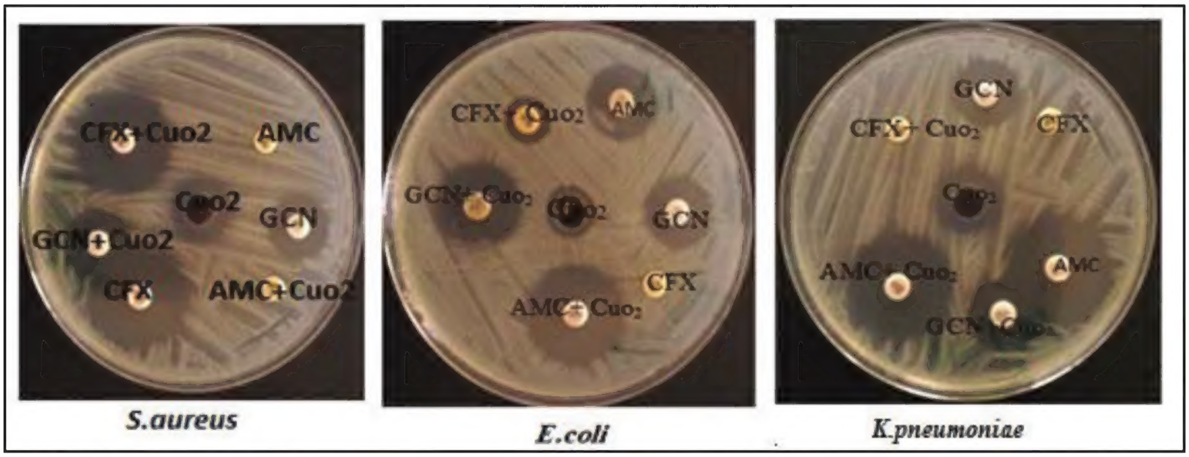

FIGURE 5. Inhibition zone of Antibiotics and complex of Antibiotics with CuONPs against some pathogenic bacteria with the present of electric fileds.

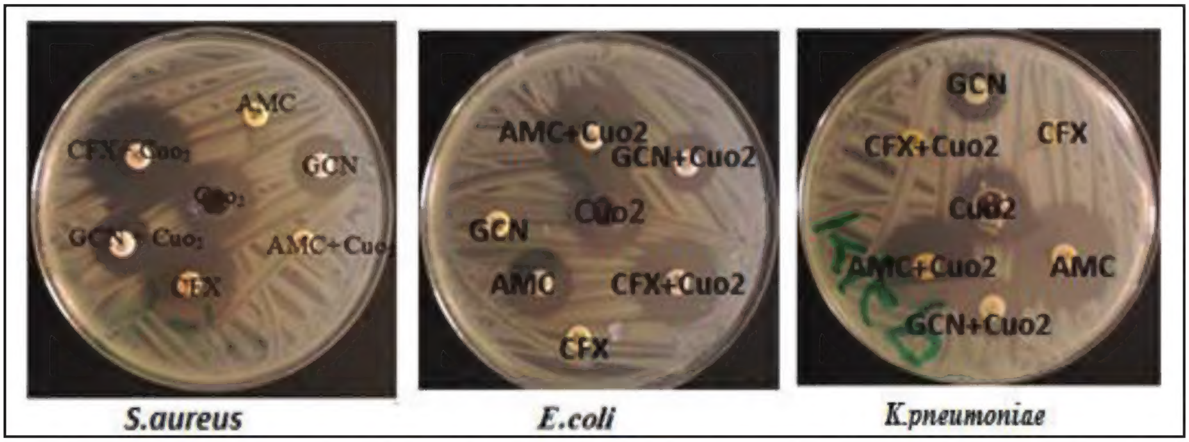

FIGURE 6. Inhibition zone of Antibiotics and complex of Antibiotics with CuONPs against some pathogenic bacteria.

In general, owing to the small size of NPs compared with bacterial pores. NPs cause damage of cell membranes by reactive oxygen species radicals. When complex of NPs and antibiotics are utilized, they have a high effect against G-*ve* and G+*ve* bacteria. This may be produced via the cell wall lysis action of antibiotics and the DNA binding action of the NPs. The antibiotic molecules have numerous active groups. Therefore, the synergistic impact can be owing to the bonding reaction with antibiotic and NPs [18].

CONCLUSION

This work demonstrated that the CuO NPs (10-100) nm can be easily generated via the laser ablation technique of immersing the metal in liquid media under electric fields. UV-Vis absorption exhibited peak of absorption spectra of CuO NPs at 251 nm. AFM results show that decrease in the particle size of CuO NPs at present of electric field. The antibacterial activity of increased with NP prepared under electric field because of their small size.

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